SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 27 October 2021

doi: 10.2903/j.efsa.2021.6936

Safety of tetrahydrocurcuminoids from turmeric (*Curcuma longa* L.) as a novel food pursuant to Regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on tetrahydrocurcuminoids from turmeric (*Curcuma longa* L.) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. Tetrahydrocurcuminoids are derivatives of curcuminoids, produced chemically by hydrogenation of curcuminoids extracted from the rhizomes of *C. longa* L. The NF consists of more than 95% of tetrahydrocurcuminoids. The applicant proposed to use the NF in food supplements at a maximum dose of 300 mg/day for adults excluding pregnant and lactating women. Taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous. There are no concerns regarding genotoxicity of the NF. Based on a 90-day oral toxicity study and a reproduction/developmental toxicity screening test performed with the NF, the Panel derives a safe level of 2 mg/kg body weight per day. For the target population this level corresponds to 140 mg/day, which is lower than the use level as proposed by the applicant. The Panel concludes that the NF, tetrahydrocurcuminoids from turmeric (*C. longa* L.), is safe for the target population at 140 mg/day.

Keywords: novel foods, tetrahydrocurcuminoids, *Curcuma longa* L., turmeric, food supplement, safety

Requestor: European Commission

Question number: EFSA-Q-2020-00111

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Declaration of Interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Suggested citation: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Bohn T, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Prieto Maradona M, Schlatter JR, van Loveren H, Ackerl R, Kouloura E and Knutsen HK, 2021. Scientific Opinion on the safety of tetrahydrocurcuminoids from turmeric (*Curcuma longa* L.) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(12):6936, 16 pp. https://doi.org/10.2903/j.efsa.2021.6936

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 3 February 2020, the company Sabinsa Europe GmbH submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283[1] to place on the EU market tetrahydrocurcuminoids from turmeric (*Curcuma longa* L.).

Tetrahydrocurcuminoids from turmeric (*Curcuma longa* L.) are intended to be used in food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council.

The applicant has requested data protection according to the provisions of Article 26 of Regulation (EU) 2015/2283.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on tetrahydrocurcuminoids from turmeric (*Curcuma longa* L.).

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA requests for supplementary information.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[2].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise: all the analytical data and certifications, two bacterial reverse mutation assays (Indian Institute of Toxicology, 2004, unpublished; Innovis, 2012, unpublished), *in vitro* micronucleus assay (Bioneeds India Private Limited, 2019, unpublished), acute toxicity study (Indian Institute of Toxicology, 2003, unpublished), full study reports of a subchronic toxicity study and a reproduction/developmental toxicity screening test (Vipragen Biosciences, 2016a,b; Majeed et al., 2019) and a human study (Sabinsa Japan Corporation, 2011, unpublished).

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only risks that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of the NF with regard to any (claimed) benefit.

3. Assessment

3.1. Introduction

The NF falls under Article 3 of Regulation (EU) 2015/2283, that is, food with a new or intentionally modified molecular structure and food consisting of, isolated from, or produced from plants and their parts.

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1–22.

[2] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

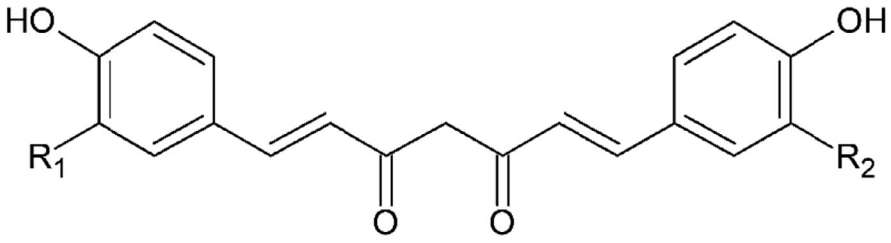
The NF which is the subject of the application is a white to pale yellow powder of tetrahydrocurcuminoids from turmeric (*C. longa* L.). The NF is produced by hydrogenation of curcuminoids from rhizomes of *C. longa* L. into tetrahydrocurcuminoids and consists of more than 95% of tetrahydrocurcuminoids. The NF is proposed to be used in food supplements. The target population is adults excluding pregnant and lactating women.

3.2. Identity of the NF

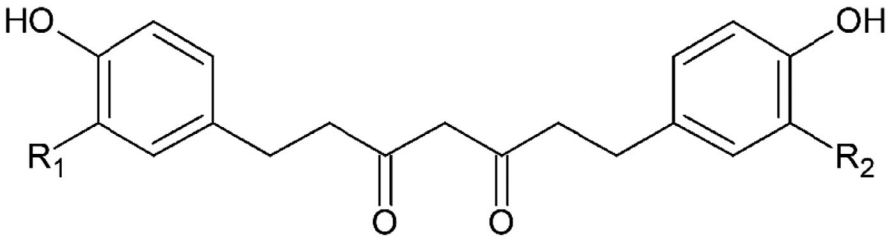
The NF is a powder of tetrahydrocurcuminoids obtained by extraction of curcuminoids from turmeric (rhizomes of *C. longa* L.) and hydrogenation into tetrahydrocurcuminoids.

C. longa is a plant of the Zingiberaceae family and rhizomes of this plant are used for the production of the NF. Common names of the plant include curcuma and turmeric. The raw material is obtained from cultivated sources. The identification of the plant material is performed based on macroscopic and microscopic characteristics of the rhizomes. Moreover, the presence of curcuminoids in ethanolic extracts of rhizomes is assessed by high-performance thin-layer chromatography (HPTLC).

Curcuminoids are constituted of curcumin (1*E*,6*E*)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (I), desmethoxycurcumin ((1*E*,6*E*)-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) (II) and bisdesmethoxycurcumin ((1*E*,6*E*)-1,7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione) (III) with curcumin being the major compound, approximately 90% of the curcuminoid content in turmeric (EMA, 2018). Upon hydrogenation, curcuminoids are converted into tetrahydrocurcuminoids, which are the colourless hydrogenated compounds and specifically tetrahydrocurcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)heptane-3,5-dione), MW: 372 (IV) from curcumin, tetrahydrodesmethoxycurcumin 1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-1,6-heptane-3,5-dione, MW: 342 (V) from desmethoxycurcumin and tetrahydrobisdesmethoxycurcumin (1,7-bis(4-hydroxyphenyl)-heptane-3,5-dione), MW: 312 (VI) from bisdesmethoxycurcumin. The identity of the three main constituents was demonstrated by HPLC/UV chromatography, ^{1}H-NMR, ^{13}C-NMR spectroscopy and mass spectrometry (Figure 1).



I. $C_{21}H_{20}O_6$ R_1 = R2 = OCH3 CAS Registry Number 458-37-7
II. $C_{20}H_{18}O_5$ R1 = OCH3, R2 = H CAS Registry Number 33171-16-3
III. $C_{19}H_{16}O_4$ R1 = R2 = H CAS Registry Number 33171-05-0



IV. $C_{21}H_{24}O_6$ R_1 = R2 = OCH3 CAS Registry Number 36062-04-1
V. $C_{20}H_{22}O_5$ R1 = OCH3, R2 = H CAS Registry Number 149579-07-7
VI. $C_{19}H_{20}O_4$ R1 = R2 = H CAS Registry Number 113482-94-3

Figure 1: Chemical structures, molecular formulae and CAS registry numbers of curcuminoids (I, II, III) and tetrahydrocurcuminoids (IV, V, VI)

In the application dossier the NF is also denominated as Curcumin C3 Reduct®.

3.3. Production process

According to the information provided, the NF is produced in line with Good Manufacturing Practice (GMP), and food safety system 22000 (International Organization for Standardization (ISO) ISO 22000:2005; ISO TS 22002-1:2009 and Additional FSSC 22000 requirements V4.1). Information on the applied Hazard Analysis Critical Control Points (HACCP) plan was also provided.

The raw material, turmeric rhizomes, is initially cleaned with water, steamed and sun dried. Then turmeric rhizomes are pulverised and pelletised. Powder of rhizomes of *C. longa* is extracted to obtain an extract rich in curcuminoids (> 95% w/w dry basis). The Panel notes that the extract rich in curcuminoids is obtained following the traditional process used for the production of the food additive curcumin E 100. The extract is further concentrated and allowed to crystallise. Crystals are filtered, washed, filtered again and dried (loss on drying < 2%). Thereafter, the curcuminoids in the extract are hydrogenated into tetrahydrocurcuminoids, using palladium on carbon (Pd/C) as a catalyst. The solution is concentrated, allowed to crystallise, filtered and dried until loss on drying < 1%. The final dried material is milled, blended, sifted and packed into bags.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3.4. Compositional data

The NF consists of more than 95% of tetrahydrocurcuminoids and in particular tetrahydrocurcumin, tetrahydrodesmethoxycurcumin and tetrahydrobisdesmethoxycurcumin.

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain required characteristics, the applicant provided analytical information for ten batches of the NF (Table 1).

Table 1: Batch to batch analysis of the NF

Parameter (Unit)	Batch number										Method of analysis
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
Total tetrahydrocurcuminoids (% w/w dry basis)	95.73	95.77	96.23	95.42	95.18	97.83	95.95	95.8	96.8	95.41	HPLC-UV (In-house method)
Tetrahydrocurcumin (% w/w dry basis)						87.38	85.02	86.51	88.38	87.29	HPLC-UV (In-house method)
Tetrahydrodesmethoxycurcumin (% w/w dry basis)						6.23	6.92	6.71	6.12	6.16	HPLC-UV (In-house method)
Tetrahydrobisdesmethoxycurcumin (% w/w dry basis)						0.24	0.38	0.36	0.20	0.21	HPLC-UV (In-house method)
Loss on drying (% w/w)	0.09		0.21		0.32						Gravimetry (UPS 731)

Parameter (Unit)	Batch number										Method of analysis
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
Moisture (% w/w)						0.35	0.35	0.25	0.19	0.34	Titration (UPS 921)
Ash (% w/w)	0.06	0.05		0.06	0.8	0.04	0.04	0.05	0.03	0.05	Gravimetry (Ph. Eur. 2.4.16 Method-II)
Palladium (mg/kg)						1.58	3.52	0.71	0.40	0.05	ICP-MS (In-house)
Lead (mg/kg)	0.35	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	ICP-OES (In-house)
Arsenic (mg/kg)	0.35	0.67	0.56	0.69	0.6	0.26	0.31	0.29	0.26	0.23	ICP-OES (In-house)
Cadmium (mg/kg)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	ICP-OES (In-house)
Mercury (mg/kg)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	ICP-OES (In-house)
Ethyl alcohol (mg/kg)						3,744	2,579	3,211	4,201	2,886	GC-HSS (UPS 467)
Acetone (mg/kg)						99.6	62.1	56.0	49.6	12.6	GC-HSS (UPS 467)
Ethyl acetate (mg/kg)						4.1	4.3	4.0	3.9	6.1	GC-HSS (UPS 467)
Isopropanol (mg/kg)						4.5	< 4.0	8.2	6.9	5.2	GC-HSS (UPS 467)
Total aerobic microbial count (CFU/g)	< 100	< 100	< 100	< 100	< 100	20	10	20	10	10	Plate Counting (USP2021, Ph. Eur. 2.6.31)
Total yeasts/moulds count (CFU/g)	< 10	10	20	10	< 10	< 10	< 10	< 10	< 10	< 10	Plate Counting (USP2021, Ph. Eur. 2.6.31)
***Escherichia coli* (per 1 g)**	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Qualitative method (USP 2022, Ph. Eur. 2.6.31)

Parameter (Unit)	Batch number										Method of analysis
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
***Salmonella* (per 25 g)**	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Qualitative method (USP 2022, Ph. Eur. 2.6.31)
***Staphylococcus aureus* (per 1 g)**	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Qualitative method (USP 2022, Ph. Eur. 2.6.13)
***Pseudomonas aeruginosa* (per 1 g)**	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Qualitative method (USP 62, Ph. Eur. 2.6.13)
Bile-tolerant Gram-negative bacteria (per 1g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Most Probable Number method (USP 2021, Ph. Eur. 2.6.31)
Coliforms (CFU/g)					< 10	< 10	< 10	< 10	< 10	< 10	Plate Counting (BAM 2001, 8th edition, chapter 4)

HPLC-UV: high-performance liquid chromatography-ultraviolet; ICP-MS: inductively coupled plasma-mass spectrometry; ICP-OES: inductively coupled plasma-optical emission spectrometry; GC-HSS: gas chromatography-headspace sampler; USP: US Pharmacopeia; Ph. Eur.: European Pharmacopoeia; BAM: Bacteriological Analytical Manual; CFU: colony forming units.

The compositional data on residual solvents (ethyl acetate, acetone, ethanol and isopropanol-Table 1) were found in compliance with Directive 2009/32[3]. Heavy metals (lead, cadmium and mercury-Table 1) were found to comply with regulatory limits as set in Regulation (EC) No 1881/2006[4]. In addition, analysis of mycotoxins was presented for five batches of the NF, including aflatoxins B1, B2, G1, M1, ochratoxin A, zearalenone and fumonisin B1 and B2 which were analysed and found below regulatory limits as set for various food categories in Regulation (EC) No 1881/2006.

Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

The Panel considers that the information provided on the composition is sufficient for characterising the NF.

3.4.1. Stability

The applicant used three independently produced batches of the NF to perform stability tests. The tests were carried out at normal storage conditions at 30°C and at 65% RH for a period of 60 months

[3] Directive 2009/32/EC of the European Parliament and of the Council of 23 April 2009 on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients. OJ L 141 6.6.2009, p. 3.

[4] Commission Regulation (EU) No No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364 20.12.2006.

and at accelerated conditions, at 40°C and at 75% RH, for a period of 6 months. The parameters analysed over the course of the stability studies were loss on drying, total tetrahydrocurcuminoids and microbiological parameters (total aerobic microbial count, total yeast and moulds count, *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and bile-tolerant Gram-negative bacteria).

For all batches analysed at normal and accelerated conditions, no changes were observed for all analysed parameters during the course of the studies. Based on the presented results, the NF does not raise any concern on stability, when packed in food grade sealed containers.

The Panel considers that the data provided sufficient information with respect to the stability of the NF during 60 months of shelf-life at normal storage conditions.

3.5. Specifications

The specifications of the NF are presented in Table 2.

Table 2: Specifications of the NF

Description: Creamy white to pale yellow powder of > 95% tetrahydrocurcuminoids obtained by hydrogenation of curcuminoids	
Source: rhizomes of *Curcuma longa* L.	
Parameter (unit)	**Specification**
Total tetrahydrocurcuminoids (dry basis) (% w/w)	> 95
Moisture (% w/w)	≤ 1
Ash (% w/w)	≤ 1
Palladium (mg/kg)	< 5
Microbiological	
Total aerobic microbial count (CFU/g)	≤ 5,000
Total yeast/moulds count (CFU/g)	≤ 100
Escherichia coli (CFU/g)	< 10
Salmonella (in 25 g)	Not detected
Staphylococcus aureus (CFU/g)	< 10
Coliforms (CFU/g)	< 10

w/w: weight per weight; CFU: colony forming units.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

There is no history of use of the NF as a food.

The source, i.e. *C. longa* rhizomes, that is used for the production of the NF has a long history of use as a spice to flavour and colour food, especially in Indian curries (e.g. Teuscher, 2003; FAO, 2004; Ziegler, 2007; Prasad and Aggarwal, 2011).

Turmeric extract, produced by extraction of dried rhizomes using organic solvents, is a food additive, i.e. curcumin (E 100), authorised in the European Union[5].

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant is the general adult population excluding pregnant and lactating women.

[5] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. OJ L 295, 12.11.2011.

3.7.2. Proposed uses and use levels

The applicant intends to market the NF as a food supplement, at a maximum dose of 300 mg per day.

3.7.3. Exposure to undesirable substances

According to the specifications, the NF may contain palladium at < 5 mg/kg. At the proposed conditions of use, the intake of palladium from the NF may thus reach an amount of up to 1.5 μg per day. The Panel notes that this amount is well below the Permitted Daily Exposure (PDE) for palladium of 100 μg per day, as established by the Committee for Human Medicinal Products of the European Medicines Agency (EMA, 2019).

3.8. Absorption, distribution, metabolism and excretion (ADME)

The applicant provided one pharmacokinetic animal study with the NF (Novaes et al., 2017). In this study the NF was dissolved in a mixture of DMSO (2%) and PEG-400 (98%) and administered by gavage to male Sprague–Dawley rats (n = 3) at 500 mg/kg. Blood samples were collected at 0, 15 and 30 min, and 1, 2, 4, 6, 12, 24, 48 and 72 h post-dose. Urine samples were collected at 0, 2, 6, 12, 24, 48 and 72 h post-dose. Serum and urine samples were treated with β-glucuronidase to release any glucuronide conjugates. A rapid absorption with an average serum tetrahydrocurcumin C_{max} of 6.8 μg/mL at 1 h was observed, followed by a short elimination phase. This was followed by two smaller tetrahydrocurcumin maxima of about 1 μg/mL at 6 and 24 h, respectively. The total amount of tetrahydrocurcumin excreted in urine was 8 μg at 24 h, which did not further increase until the end of the study (72 h). The presence of tetrahydrocurcumin in urine, even though at low concentrations, indicates oral absorption to some degree and elimination at least in part through the renal route (Novaes et al., 2017).

The applicant also provided an overview of the metabolism of curcumin and curcuminoids as described in the literature. Tetrahydrocurcumin is one of the metabolites of curcumin, produced *in vivo* through Phase I metabolism in the liver by hepatic reductases. Similar to other reduced curcumin metabolites, tetrahydrocurcumin is conjugated with glucuronide (via glucuronyltransferases) or sulfate (via sulfotransferases), resulting in tetrahydrocurcumin glucuronoside (about 30% (Fança-Berthon et al., 2021)) or corresponding monosulfate. Excretion occurs primarily via the biliary system and, to a lesser extent, via the urinary system (Holder et al., 1978; Heger et al., 2014; Jäger et al., 2014). Based on this information, it can be assumed that the NF is mainly excreted via the bile.

3.9. Nutritional information

The applicant provided nutritional information for five batches of the NF. Small quantities of fats (0.22–0.29%), moisture (0.19–0.35%), ash (0.03–0.05%) and sodium (0.001–0.002%) were detected. Proteins and carbohydrates (including fibre and starch) were not detected (however, limits of detection for proteins and carbohydrates, 0.27 and 2.7 g/kg, respectively, were high).

The Panel considers that, taking into account the composition of the NF, which consists of > 95% tetrahydrocurcuminoids, and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided six toxicological studies. These studies, which were claimed proprietary by the applicant, are listed in Table 3.

Table 3: List of toxicological studies with the NF

Reference	Type of study	Test system	Dose
Indian Institute of Toxicology (2004, unpublished)	Bacterial reverse mutation test (GLP, OECD TG 471)	*Salmonella* Typhimurium	Up to 5,000 μg/plate (in presence and absence of S9 mix)
Innovis (2012, unpublished)(1)	Bacterial reverse mutation test (GLP, OECD TG 471)	*Salmonella* Typhimurium	Up to 1,250 μg/plate (in presence and absence of 3.10.S9 mix)
Bioneeds India Private Limited (2019, unpublished)	*In vitro* mammalian erythrocyte micronucleus test (GLP, OECD TG 487)	Human lymphocytes	UP to 250 μg/mL
Indian Institute of Toxicology (2003, unpublished)	Acute oral toxicity study	Sprague Dawley rats	2,000 mg/kg bw per day
Vipragen Biosciences (2016a, unpublished), Majeed et al. (2019)	90-day repeated dose oral toxicity study with a 14-day recovery period (GLP, OECD TG 408)	Wistar rats	up to 400 mg/kg bw per day
Vipragen Biosciences (2016b, unpublished), Majeed et al. (2019)	Reproduction/developmental toxicity screening test (OECD TG 421)	Wistar rats	up to 400 mg/kg bw per day

bw: body weight; GLP: Good Laboratory Practice; OECD TG: Organisation for Economic Co-operation and Development test guidelines.
(1): Isopropyl alcohol was used instead of ethyl acetate for the manufacturing process of the test item.

The studies were conducted in accordance with respective OECD (Organisation for Economic Co-operation and Development) test guidelines (TG) No 471, 487, 408, 421 (OECD, 1997, 2016a,b, 2018) and in compliance with principles of Good Laboratory Practice (GLP) (OECD, 1998). For one bacterial reverse mutation assay (Indian Institute of Toxicology, 2004, unpublished) no GLP certificate but a signed statement of compliance with GLP was provided by the laboratory, which appears to be accredited by NABL (National Accreditation Board for Testing and Calibration Laboratories, India).

3.10.1. Genotoxicity

The applicant submitted two bacterial reverse mutation tests (Indian Institute of Toxicology, 2004, unpublished; Innovis, 2012, unpublished) and one *in vitro* mammalian erythrocyte micronucleus test (Bioneeds India Private Limited, 2019, unpublished). The full study reports and certificates of analysis of the test items were provided.

In the originally submitted bacterial reverse mutation test (Innovis, 2012, unpublished), a test item was used which was produced by a manufacturing process that is not fully in compliance (i.e., isopropyl alcohol was used instead of ethyl acetate) with the production process of the NF as presented under section 3.3. Concentrations up to 1,250 μg/plate were tested for potential mutagenicity using the *Salmonella* Typhimurium tester strains TA98, TA100, TA102, TA1535 and TA1537. The highest dose of 1,250 μg/plate was chosen based on solubility and precipitation tests. There were no increases in the number of revertant colonies, neither in the presence or absence of metabolic activation, in both confirmatory trials (plate incorporation method and pre-incubation method, respectively).

Following an EFSA request for additional information and clarification on the test item in the study described above, the applicant submitted an additional bacterial reverse mutation test (Indian Institute of Toxicology, 2004, unpublished). In this test, the NF (containing 95.3% tetrahydrocurcuminoids) was tested at various concentrations up to 5,000 μg/plate. The *S*. Typhimurium tester strains TA97a, TA98, TA100, TA1535 and TA102 were used, and the plate incorporation method was applied. The NF up to the highest concentration tested did not induce increases in revertant colonies, with or without metabolic activation (S9-mix).

The *in vitro* mammalian erythrocyte micronucleus test (Bioneeds India Private Limited, 2019, unpublished) was performed in human lymphocytes with a batch of the NF that contained 95.1% tetrahydrocurcuminoids. In the initial cytotoxicity test, the test item was applied at concentrations of 0.0625, 0.125, 0.25, 0.5 and 1 mg/mL. As there was excessive cytotoxicity (84.8% and 96.7%,

respectively) observed at the two highest concentrations, these were not applied in the main experiment. Thus, concentrations of 0.0625, 0.125 and 0.25 mg/mL were used (for short term treatment in the presence and absence of metabolic activation (S9-mix), for long term treatment in the absence of metabolic activation). At the highest dose (i.e. 0.25 mg/mL) tested in the main experiment, cytotoxicity amounted to 38.3% (short term treatment plus S9-mix), 43.3% (short term treatment without S9-mix) and 41.7% (long term treatment without S9-mix). There was no increase in the percentage of micronuclei at any of the tested concentrations compared to the vehicle control, both with and without metabolic activation.

Taking into account the test results provided, the Panel considers that there are no concerns regarding genotoxicity of the NF.

3.10.2. Acute and subchronic toxicity

The summary report of an acute oral toxicity study was provided (Indian Institute of Toxicology, 2003, unpublished), for which Sprague–Dawley rats (5/sex) received once by gavage 2,000 mg/kg body weight (bw) of the NF. All animals survived the 14-day observation period. The Panel considers that acute toxicity studies are, in general, of limited relevance for the safety assessment of NFs.

A 90-day repeated dose oral toxicity study with the NF was provided (Vipragen Biosciences, 2016a (unpublished study report); Majeed et al., 2019). The study included a 14-day recovery period. Adult Wistar rats (10/group and sex for the main groups; 5/group and sex for the recovery groups) were administered by gavage 0 (vehicle only), 100, 200 or 400 mg/kg bw per day of the NF for 90 days. The batch of the NF that was used for this study contained 95.4% tetrahydrocurcuminoids. Carboxy methyl cellulose (0.5%) was used as a vehicle. The rats in the recovery group were kept for 14 days after termination of the treatment.

There was no mortality nor were there any clinical signs observed in any of the study groups during treatment and recovery period. There were no differences in body weight, body weight gain or food consumption between the groups treated with the NF and the control groups. There were no changes in the ophthalmological examination. There were no differences in absolute or relative organ weights between the treated and the control groups. The animals appeared normal from day 1 to the end of observation and did not reveal any functional deficits.

A number of statistically significant differences between control groups and groups that received the NF were observed in the study, which are briefly summarised as follows. A lower blood platelet count was seen in males in the high-dose group compared to the control group. Lymphocytes and monocytes were higher in females in the mid- and high-dose groups when compared to controls, while in males monocytes were lower in the mid- and high-dose groups (compared to controls). Total cholesterol was higher in the high-dose group in males while in females triglycerides were lower in the low-dose group, compared to controls. The Panel considers that the observed differences were small, partly without a clear dose–response relationship, and that they reflect normal biological variation rather than adverse effects.

There were no findings in gross pathology or histopathology, apart from those commonly observed in this rat strain at this age and which were of similar incidence and severity in control and treated animals.

The Panel considers that the no observed adverse effect level (NOAEL) of this study is the highest dose tested, i.e. 400 mg/kg bw per day.

3.10.3. Reproductive and developmental toxicity

A reproduction/developmental toxicity screening test according to OECD TG 421 was provided (Vipragen Biosciences, 2016b (unpublished study report); Majeed et al., 2019).

Adult Wistar rats (10/group and sex) were assigned to four groups that received by gavage 0 (vehicle only), 100, 200 or 400 mg/kg bw per day of the NF. The batch of the NF that was used for this study contained 95.4% tetrahydrocurcuminoids (same batch as for the above 90-day study). The males were administered the NF for 2 weeks prior to mating, during the mating period and thereafter until a total dosing period of 43 days. The females were dosed for 2 weeks prior to mating, through mating, pregnancy and up to lactation day 3. On lactation day 4, pups and dams were sacrificed.

There were no clinical signs or mortality during the study apart from one female in the mid-dose group, which was found dead on gestation day 23 due to dystocia. The mean body weights for males throughout the study and for females up to day 15 did not differ as compared to the control groups. However, during gestation maternal body weight in the high-dose group was statistically significantly

lower (by 6.6–8.6%) than in controls. With respect to food consumption, some statistically significant differences in different directions were observed between groups during some weeks, however without corresponding changes observed in body weights, and hence were not considered relevant.

The mean number of pups, mean body weights of pups, mean litter size, mean viable litter size and day 4 survival index did not significantly differ between the groups. There were no external abnormalities in live or dead pups, apart from tail deformity observed in 9 out of 10 pups of one dam in the low-dose group. This was, however, an isolated finding not observed in the mid- or high-dose group. There were no statistically significant differences for the pre-coital interval, gestation length, male fertility, mating index, female fertility and mating and fecundity index in any of the treated groups when compared to the control groups.

The findings observed in histopathology of reproductive organs and adrenals were of similar incidence and severity in control and treated animals.

Even though reported as not statistically significant, the Panel notes a higher mean post-implantation loss (difference between implantations and live foetuses/pups in percent) of 30.0% in the high-dose group as compared to 15.2%, 12.9% and 16.7% in control, low-dose and mid-dose groups, respectively. The Panel also notes a lower live birth index of 83.1% in the high-dose group as compared to 95.8%, 100% and 98.9% in control, low-dose and mid-dose groups, respectively. This lower live birth index was owing to 14 dead pups (from 4 dams) in the high-dose group, versus 4 dead pups in the control group, 0 in the low-dose group and 1 in the mid-dose group. The Panel considers that these findings may point towards adverse effects of the NF in the high-dose group and, therefore, sets the NOAEL of this study at the mid-dose, i.e. 200 mg/kg bw per day.

3.10.4. Human data

The applicant submitted one open-label, uncontrolled (single arm) study in 20 subjects that received 300 mg (one tablet) of the NF per day for 28 days (Sabinsa Japan Corporation, 2011, unpublished). According to the provided certificate of analysis of the study item, the 300 mg contained 292.2 mg tetrahydrocurcuminoids. All the study participants (5 females and 15 males; mean age 28.3 years; mean body mass index (BMI) = 23.8 kg/m^2) completed the study. The endpoints of the study included blood pressure, body weight/BMI, haematology, clinical chemistry and recording of adverse events. No relevant (or statistically significant) changes were found at 28-days compared to baseline. No adverse events were reported during the study. The Panel considers that this uncontrolled study is of little value for the safety assessment of the NF.

3.11. Allergenicity

The NF consists of > 95% of tetrahydrocurcuminoids. Protein was not detected (at a limit of detection of 0.27 mg/g) in the analysis submitted for five batches of the NF.

The Panel considers that the risk of allergic reactions to the NF is low.

4. Discussion

The NF which is the subject of the application is tetrahydrocurcuminoids (> 95%) from turmeric (*C. longa* L.). The main derivative is tetrahydrocurcumin (average percentage 87%) while tetrahydrodesmethoxycurcumin and tetrahydrobisdesmethoxycurcumin are present in lower amounts (average percentages of 6.4% and 0.3%, respectively).

The applicant intends to market the NF as a food supplement at a maximum dose of 300 mg per day. The target population is adults excluding pregnant and lactating women.

Taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous.

The Panel considers that the NOAEL of the provided 90-day oral toxicity study is the highest dose, i.e. 400 mg/kg bw per day, tested in the study. By applying an uncertainty factor of 200 (10 (interspecies variability) × 10 (intraspecies variability) × 2 (subchronic to chronic study duration)), the Panel derives a safe level of 2 mg/kg bw per day.

The applicant also submitted a reproduction/developmental toxicity screening test, for which the Panel identifies a NOAEL of 200 mg/kg bw per day (i.e. mid-dose as tested in the study). For this study, the Panel considers an uncertainty factor of 100 (10 (interspecies variability) × 10 (intraspecies variability)) as adequate since no adjustment for study duration is required. The Panel thus derives a

safe level of 2 mg/kg bw per day, which is identical to the safe level based on the 90-day toxicity study.

For the target population (adults excluding pregnant and lactating women) with a default body weight of 70 kg (EFSA Scientific Committee, 2012), the safe level of 2 mg/kg bw per day corresponds to 140 mg of the NF per day, which is lower than the use level as proposed by the applicant.

5. Conclusions

The Panel concludes that the NF, tetrahydrocurcuminoids from turmeric (*C. longa* L.), is safe for the target population at 140 mg/day.

5.1. Request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the following data claimed as proprietary by the applicant: analytical data, bacterial reverse mutation test (Indian Institute of Toxicology, 2004, unpublished), *in vitro* micronucleus assay (Bioneeds India Private Limited, 2019, unpublished), full study reports of the subchronic toxicity study and the reproduction/developmental toxicity screening test (Vipragen Biosciences, 2016a,b; Majeed et al., 2019).

6. Steps taken by EFSA

1) On 29/07/2020, EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of tetrahydrocurcuminoids from turmeric (*Curcuma longa* L.). Ref. Ares(2020)4001815.
2) On 29/07/2020, a valid application on tetrahydrocurcuminoids from turmeric (*Curcuma longa* L.), which was submitted by Sabinsa Europe GmbH, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/1526) and the scientific evaluation procedure was initiated.
3) On 20/11/2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 05/05/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 23/07/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
6) On 10/09/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) During its meeting on 27/10/2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of tetrahydrocurcuminoids from turmeric (*Curcuma longa* L.) as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
BAM	Bacteriological Analytical Manual
BMI	body mass index
bw	body weight
CAS	Chemical Abstracts Services
CFU	colony forming units
^{13}C-NMR	carbon nuclear magnetic resonance
CoA	certificate of analysis
DMSO	dimethyl sulfoxide

EMA	European Medicines Agency
FAO	Food and Agriculture Organization
FSSC	Food Safety System Certification
GC-HSS	gas chromatography–headspace sampler
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
^{1}H-NMR	Proton nuclear magnetic resonance
HPLC-UV	high-performance liquid chromatography-ultraviolet
HPTLC	high-performance thin-layer chromatography
ICP-MS	inductively coupled plasma-mass spectrometry
ICP-OES	inductively coupled plasma-optical emission spectrometry
ISO	International Organization for Standardization
LoD	limit of detection
MW	molecular weight
NABL	National Accreditation Board for Testing and Calibration Laboratories
NDA	Panel on Nutrition, Novel Foods and Food Allergens
NOAEL	no observed adverse effect level
NF	novel food
OECD	Organisation for Economic Co-operation and Development
Pd/C	palladium on carbon
PDE	permitted daily exposure
PEG	polyethylene glycol
Ph. Eur.	European Pharmacopeia
RH	relative humidity
TG	test guidelines
USP	US Pharmacopeia
w/w	weight per weight